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User's Manual and Instructions

ELISA Kit for Antibody to Hepatitis B Surface Antigen

Catalog No.: KO31004096

INTENDED USE

ELISA Kit for Antibody to Hepatitis B Surface Antigen is an *in vitro* enzyme immunoassay for the detection of Anti-HBs in human serum or plasma.

PRINCIPLE

The purified HBsAg is coated on the solid phase of multi-wells. Serum sample and Horseradish peroxidase labeled with HBsAg (conjugated) are added to coated wells. After incubation, if Anti-HBs is present in the sample, a complex of HBsAg -Anti-HBs-HBsAg labeled with HRP will form. Wash wells to remove other unbounded serum components, incubate with substrates (TMB) to form a colored product, and measure the absorbance at 450nm to indicate the presence or absence of Anti-HBs in the sample. The test is special, sensitive, reproducible and easy to operate.

STORAGE AND STABILITY

Store the kit at 2~8℃. The kit is stable unopened within 12 months after it is received.

MATERIALS PROVIDED

1. HBsAg Coated Microwell Plate	1 block (96wells)
2. Enzyme Conjugant	1 bottle (6.5ml)
3. Positive Control Serum	1 vial (1.0ml)
4. Negative Control Serum	1 vial (1.0ml)
5. Wash Buffer (1:20 dilution prior to use)	1 bottle (50ml)
6. Substrate A	1 bottle (8.5ml)
7. Substrate B	1 bottle (8.5ml)
8. Stop Solution	1 bottle (8.5ml)
9. Seal Paper	2 pieces

[PRECAUTIONS]

1. The samples should be fresh and avoid hemolysis, bacterial growth, and repetitive freeze-thaw cycle.
2. Do not interchange reagents between different lots.
3. The seal paper CANNOT be used repeatedly.
4. Mix reagents well before use. If crystal form in certain reagents, such as wash buffer, warm bottle/vials to redissolve. Mix well.
5. Follow the instruction provided in the manual, especially for incubation temperature and reaction time. All pipetting devices should be used with care and calibrated regularly following the manufacturer's instructions.
6. Put the remaining reagents in the sealed pouch, and return them to 2~8°C for short period of storage.
7. To prevent cross-contamination, wear gloves and lab coats throughout the procedure, and disinfection spills immediately. Dispose of all samples and materials used to perform the test. To disinfect used samples and materials before disposal, one should autoclave at 121°C or use 5.0 g/L liquid sodium hypochlorite solution (The positive control serum in the kit has been inactivated already).

ASSAY PROCEDURE

1. For each test, set one blank, two positive and two negative controls, add 0.05ml serum sample, positive and negative control serum into the coated wells, then add one drop (approximately 0.05ml) of enzyme conjugant into the same coated wells (The blank well is omitted), mix thoroughly, cover wells with seal paper, and incubate for 30-60 minutes at 37℃.

2. **Manual Wash Procedure:** Discard the liquid in the coated wells and bring them to dry. Fill the wells with 300ul wash buffer, discard the liquid. Repeat 5 times and then bring them to dry.
   Automatic Wash Procedure: Select the automatic operations of washing 5 times and bring them to dry after the operation.

3. Add one drop (approximately 0.05ml) of substrate A and B respectively to each well, seal the plate with seal paper, mix thoroughly, and incubate for 10-15 minutes at 37℃. Avoid exposure to light.

4. Add one drop (approximately 0.05ml) of stop solution into each well, mix thoroughly to terminate the reaction. Measure the absorbance at 450nm against the blank or measure the absorbance at 450nm/630-690 nm.

INTERPRETATION OF RESULTS

Colorimetric Method
Cut Off Value calculation:
COV = the average OD of negative controls × 2.1

Positive	OD_{450} of sample ⩾ COV
Negative	OD_{450} of sample ＜ COV
Invalid	If the OD of positive control is ≤ 1.0 or negative control is ≥ 0.1, the result is invalid. In any event, repeat the test. If the problem persists, contact the local distributor.
Notes	If the absorbance of negative controls is below 0.05, calculate it as 0.05. If the absorbance of negative controls is above 0.05, calculate it as its original value.

PERFORMANCE CHARACTERISTICS

Sensitivity 10mIU/ml, OD⩾0.105
Specificity the average OD of 20 normal negative samples ⩽0.030
Precision CV(%)⩽15% (n=10)

This Kit is for Research Use Only